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Behavioural response of honeybees (*Apis mellifera scutellata* Lep.) to wild pollinators on sunflowers (*Helianthus annuus* L.)

by

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Submitted in partial fulfillment of the requirements for the degree
Master of Science (Agric): Entomology

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Declaration

I, Awraris Getachew Shenkute, declare that the thesis, which I hereby submit for the degree Master of Science (Agric): Entomology at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature: _______________

April 2010

Dedication

I dedicate this thesis to my mother, who had bright hope to see my success. I wish she had seen the fruit of her effort.

Table of contents Page

List of tables

List of figures

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Behavioural response of honeybees (*Apis mellifera scutellata* Lep.) to wild pollinators on sunflowers (*Helianthus annuus* L.)

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Degree: Master of Science (Agric): Entomology

Abstract

Pollination is an essential ecosystem service, increasing reproductive success of many crops, which can be provided by managed pollinators, wild bees (including honeybees) and other insect pollinators. However, the pollination services and the economic value of wild pollinators are often underestimated. Better understanding of the factors that influence honeybee foraging behaviour and pollination efficiency can contribute to the improvement of management practices that aim to enhance crop pollination and ecosystem services.

The objectives of this study were to investigate the importance of managed honeybees and wild honeybees to sunflower pollination as well as to evaluate the response of honeybees to different levels of floral rewards and to behavioural interactions with wild flower visitors. The study was conducted in 16 commercial sunflower farms and one experimental farm of South Africa during the 2009 sunflower flowering season.

The results showed that insects, particularly honeybees, were efficient pollinators, improving sunflower production in all self-fertile sunflower cultivars used in this study. Furthermore, wild honeybee colonies were found to be as efficient as managed honeybee colonies in sunflower pollination near to natural habitat. Both sunflower yield and the abundance of pollinators decreased with distance from natural habitat, suggesting that sunflower yield is directly correlated with the abundance of pollinators. The amount of nectar present in the florets of sunflower significantly affected pollinator behaviour, influencing honeybee visitation length and foraging rate which prefer to exploit floral rewards from the same source if they find the higher amount per foraging trip, possibly having a negative impact on cross-pollination. Moreover, the concentration of nectar collected from honeybees was significantly lower than the nectar concentration from florets, suggesting that honeybees diluted highly concentrated sunflower nectar with their saliva to their optimum concentration level.

Interspecific exploitative competition between honeybees and wild pollinators (wild bees, butterflies and moths) significantly increased the movement of honeybees among sunflower heads, which enhances cross-pollination. Furthermore, behavioural interactions influenced the length of foraging time spent by individual honeybees per sunflower head. Butterflies were the most influential in enhancing honeybee foraging movement, followed by wild bees and then moths. The importance of a given flower visitor species to honeybee movement is likely related to the size of the visitor, as the bigger size of butterflies and movement of their wings increases the chance of disturbing a neighbouring honeybee.

Conservation of natural habitat is important to maintain the diversity of flower visitors which indirectly contribute to crop production by enhancing honeybee foraging activity and consequent direct pollination service. Furthermore, the pollination effectiveness of wild pollinators, density of wild honeybees surrounding sunflower fields and effects of human activities on pollination disruption are suggested as topics for future research.

CHAPTER 1

General introduction

Pollination as an ecosystem service

Pollination is a vital ecosystem service, increasing reproductive success of many crops (FAO, 2004). This service can be provided by wild and managed honeybees, other wild bees or other insect pollinators in general (Cunningham *et al.*, 2002). The term wild honeybee is used to describe those unmanaged honeybees living in natural vegetation like woodlots, windbreaks, wastelands and parks (Chang and Hoopingarner, 1991) when indigenous to an area. If introduced to a new country, wild colonies are termed feral. Managed honeybees are kept in bee hives for honey and wax production, as well as for pollination services. Over 80% of plant pollination is carried out by insects, and bees contribute nearly 80% of the total insect crop pollination (Johannsmeier and Mostert, 2001; Thapa, 2006). One-third of the total human diet in the USA is dependent directly or indirectly, on insect pollinated plants (McGregor, 1976; Richards and Kevan, 2002), with most of the world's diet of vegetable fats and oils coming from crops which depend on insect pollination (rape seed, sunflower, peanuts, cotton and coconuts; see Richards and Kevan, 2002).

Reproductive output of plants depends not only on the visitation rate of pollinators but also on the amount of viable pollen transferred to the stigma, and this depends on the type of insect visitors and their foraging behaviour (Davila and Wardle, 2008). Honeybees are active and regular foragers due to their own nutritional needs and those of their progeny (Kearns and Inouye, 1997), and subsequently are considered to be the best pollinators (Thapa, 2006; Greenleaf *et al.*, 2007; Brosi *et al.*, 2008). However, honeybee pollination service can provide maximum seed set and fruit production in shorter foraging distances from their nest (Richards and Kevan, 2002) for some crops.

Insect pollinators are negatively affected by habitat fragmentation in intensive agricultural landscapes (Öckinger and Smith, 2007), particularly when fields are isolated from natural and semi-natural vegetation (Kremen *et al*., 2004; Greenleaf and Kremen, 2006b; Ricketts *et al*., 2008), potentially decreasing crop pollination success (Steffan-Dewenter and Tscharntke, 1999; Cunningham, 2000; Aguilar *et al*., 2006; Albrecht *et al*., 2007). To maintain sustainable insect pollination services it is essential to conserve flower-rich natural habitats (Steffan-Dewenter and Tscharntke, 1999; Westphal *et al*, 2003; Öckinger and Smith, 2007). In addition, due to the absence of insecticide applications, bee diversity is generally found to be higher in organic farming systems compared to conventional farms (Holzschuh *et al*., 2007).

Foraging behaviour of flower visitors

Flower characteristics can affect insect foraging behaviour (Thapa, 2006, Stang *et al*., 2009). Foraging honeybees generally collect food resources from flowers until the maximum transportable amount has been collected (Raw, 2000). Honeybees prefer to visit flowers of the same patch until a more profitable food source becomes available (Ribbands, 1949; Du Toit, 1988; Goulson, 1994; Johannsmeier and Mostert, 2001) which makes them very efficient pollinators (Du Toit, 1988; Free, 1993). The time spent per flower depends on the quantity of resources available, the distance the bees need to travel to forage, weather conditions and interspecific competition (Neff and Simpson, 1990; Richards and Kevan, 2002). When resources are scarce honeybees become more restless, so that instead of visiting flowers quite close to each other they jump from one portion of their foraging area to another (Ribbands, 1949).

Some flowers are not the proper size for honeybees to enter, while others have little nectar making them unattractive, or specialized mechanisms for pollen release that can not be operated by honeybees (Kearns and Inouye, 1997). Thus for some crops looking at pollinators other than honeybees is important. Previous studies have found that several crops are effectively visited by wild bees (non-*Apis* species) (Eardley and Mansell 1993; 1996; Raw, 2000; Kremen *et al*., 2002; Greenleaf and Kremen, 2006a; Winfree *et al*., 2008; Macias *et al*., 2009), their pollination service being equivalent to that of managed honeybee pollinators under favourable circumstances (Kremen *et al*., 2002) and effective in maintaining the crop’s genetic diversity (Raw, 2000).

Sunflower pollination

The morphology of sunflower (*Helianthus annuus* L.) florets makes self fertilization impossible until late flowering stages (Free, 1964), and cross pollination is favoured by self-incompatible mechanisms (Free, 1993; Butz Huryn, 1997). The presence of bright yellow ray florets on sunflowers serves as a visual attraction for insects (Du Toit, 1988; Neff and Simpson, 1990), while the disk florets provide a highly clumped energy reward and crowding of the florets ensures that a maximum number of florets is pollinated by a single insect visit (Du Toit, 1988). Honeybees do not visit ray-less sunflower heads (Neff and Simpson, 1990). With an adequate number of pollinators present and good transfer of pollen, the stigma is pollinated within the first 24 hours after it becomes receptive for pollen (Du Toit, 1988). When unfertilized it may remain at the female stage for a period of 10 days or longer (Neff and Simpson, 1990; Free, 1993); after that it reaches a size of twice its normal length which results in unfertilized seeds (Du Toit, 1988).

Honeybee pollination increases seed production and oil content of *Helianthus annuus* seed (Free, 1993). Managed honeybees are the major pollinators of commercial sunflowers in South Africa, although other insects may also contribute (Du Toit, 1988). The body of honeybees is covered with branched hairs which make them morphologically well adapted for pollen collection, while their easy management in hives makes them economically suitable as mass crop pollinators (McGregor, 1976; Du Toit, 1988).

Honeybees belong to the group of long tongued bee species which have been able to take advantage of increasing complexity of advanced angiosperm flowers (Winston, 1987). A nectar foraging honeybee pushes its tongue and head between the petals and anther tube to suck the nectar from the base of the corolla, and often becomes highly dusted with pollen (Free, 1993). Long tongued anthophorid bees are able to exploit the nectar from all sunflower cultivars while short tongued halictid bees can not reach the nectar and forage only for pollen on the heads (Du Toit, 1988). Nectar collecting honeybees that discard pollen may cause pollination indirectly with the pollen they discard while hovering over a flower head (Free, 1993). Moreover, they make contact with a number of stigmas on the same head while probing a single floret, due to the pumping action of the abdomen when sucking nectar, and also frequent leg movement (Du Toit, 1988), which is important to transfer pollen between different florets on the same head.

Contribution of wild pollinators

The pollination services and the economic value of wild bees are often underestimated (Kearns and Inouye, 1997; Greenleaf and Kremen, 2006 a & b). Wild bees are defined as all bees except honeybees in the genus *Apis*. According to Losey and Vaughan (2006) the pollination services provided by wild bees to United States fruit and vegetable production is estimated to be about $3.07 billion per year. Moreover, studies of hybrid sunflower pollination (male and female flowers are found in different sunflower heads) in California show that the pollination services of honeybees to set hybrid sunflower seed are greatly enhanced by behavioural interactions with wild bees, which makes honeybees move more quickly between plants in different male and female sunflower rows and collect more pollen on their bodies (Greenleaf and Kremen, 2006b). Hybrid sunflower seed is produced using blocks of male fertile rows alternating with blocks of male sterile rows which depend upon pollinators to transfer pollen from the pollen-bearing restorer lines to the male sterile female parents (Free, 1993). According to Greenleaf and Kremen (2006b) the amount of direct wild bee pollination and enhanced honeybee pollination delivered to sunflowers depends on farm management practices and the proximity to natural habitat (1-3 km radius). However, the contribution of wild bees and wild honeybees to normal (non-hybrid) sunflower pollination has not yet been clearly demonstrated. In the case of non-hybrid sunflower, male and female flowers are found on the same sunflower head. Wild honeybee colonies are generally smaller in size than managed honeybee colonies, due to the limited capacity of hollows and cavities of their nest, and hence managed honeybee colonies are likely to harvest 3-5 times the amount of food resources being collected by wild honeybee colonies (Paton, 1996). In addition, continuous removal of surplus honey and pollen from managed hives by beekeepers causes honeybees to collect more nectar and pollen resources. However, to our knowledge, no study has clearly compared the effectiveness of wild and managed honeybees as sunflower pollinators in South Africa.

Aim of this study

Increasing our understanding of honeybee activity and behaviour in sunflower fields and clarifying the contribution of different pollination vectors for production is essential to maximize their pollination function (Du Toit, 1988). Due to their high economic importance, conservation of pollination services is a reason for concern world-wide (Kearns *et al*., 1998). In South Africa there is a greater demand for commercial sunflower production as a source of vegetable oil and food than what is available (Nel, 2001; Du Toit, 1988). For example, sunflower seed production ranges between 500 000 and 700 000 tons per year (Sunflower production guideline, 2010) which is below the demand to fulfill oil requirement of the country in 2000/01 (Nel, 2001).

This study was conducted as part of the South African pollinator project in collaboration with SANBI, quantifying sunflower pollination on commercial farms in Settlers and through pollination trials at the experimental farm of the University of Pretoria. The first aim of this study was to investigate the importance of managed honeybees and wild honeybees to sunflower pollination and to evaluate the foraging activities of honeybees on sunflowers (Chapter 2). The second aim was to evaluate the response of honeybees to various levels of floral rewards and their behavioural interactions with wild flower visitors on sunflowers (Chapter 3).

The results of this MSc thesis will contribute to increase our knowledge of the pollination of this important crop, clarifying crucial aspects of the importance of natural habitats in overall pollination success.

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CHAPTER 2

Foraging behaviour and crop pollination efficiency of honeybees on sunflowers

Introduction

Pollination efficiency of an insect species is affected by its foraging behaviour (Jacobi, 2002; Young *et al*., 2007), frequency of visits (Kawai and Kudo, 2008), and the amount of pollen transferred to the stigma (Gyan and Woodell, 1987). Understanding the foraging behaviour of flower visitors is, therefore, essential for a full understanding of plant-pollinator interactions (Jacobi, 2002). This behaviour is influenced by the amount of floral rewards available, the distribution and abundance of co-flowering plants, and the presence of other flower visitors (Goulson, 1999).

Sunflower production ranks second as a source of edible oil in the world, next to soybean (De Paiva *et al*., 2003). The contribution of wind in transferring sunflower pollen is negligible (Low and Pistillo, 1986). Thus, it needs animal pollinators to be transferred from male to female flowers (Free, 1993). A frequently visited sunflower cultivar can produce significantly higher hybrid seed yield than a less visited cultivar if a sufficient amount of pollen is deposited (Skinner, 1987). Hybrid sunflower seed is produced from hybridized male-sterile and male-fertile lines (Parker, 1981). Similarly, non-hybrid sunflower seed production is enhanced by honeybee pollination (Freund and Furgala, 1982; Kamler, 1997; De Paiva *et al*., 2003; De Grandi-Hoffman and Chambers, 2006; Nderitu *et al*., 2008), leading to higher seed set and oil content as well as better germination rates (Langridge and Goodman, 1974, Freund and Furgala, 1982, De Grandi-Hoffman and Chambers, 2006). However, different cultivars may be differently affected by honeybee visitation. Of the 24 cultivars of sunflower tested for self fertility by Low and Pistillo (1986) in Australia, only two cultivars had better seed set due to their higher self fertility when honeybees were absent.

Sunflower is among the most important nectar producing crops and provides high energy rewards for bees (El-Sarrag *et al*., 1993; Zajacz *et al*., 2008) which ensures good honey production (Nicoleta *et al*, 2007). Therefore, sunflower is a good honeybee crop and other pollinators as a source of nectar (El-Sarrag *et al*., 1993; Jones and Gillett, 2005). Sunflower cultivars, however, vary in the amount of net energy provided to insect pollinators (Parker, 1981; Freund and Furgala, 1982; Tepedino and Parker, 1982), thus potentially affecting attractiveness to foraging bees (Skinner, 1987; Cnaani *et al*., 2006; Abrol, 2007). A change in the volume of floral rewards can also play an important role in determining attractiveness (Fell 1986; Shafir *et al*. 1999), with some species of bees being more sensitive to changes in volume (e.g. honeybees, see Silva and Dean, 2000) while others are more sensitive to changes in sugar concentrations (e.g. bumble bees, see Cnaani *et al*., 2006).

Better understanding of the factors that influence honeybee foraging behaviour (managed and wild) and pollination efficiency can contribute to the improvement of management practices that aim to enhance crop pollination. This study was conducted to investigate the contribution of managed honeybee colonies and wild honeybees to sunflower pollination, and to evaluate honeybee foraging behaviour as well as activities on disc florets of sunflower heads.

Materials and methods

Study sites and field design

In order to compare the contribution of managed and wild honeybees in sunflower pollination, observations on the foraging behaviour of honeybees were conducted on seven commercial sunflower fields located in Limpopo Province, South Africa (27^0 57'S, $28^0$32'E), during the 2009 sunflower flowering season (17 March - 08 April). Fields were divided in two categories, according to sources of flower visitors: i) fields next to natural habitat and without managed honeybees, their natural margins being a source of wild flower visitors (four fields), and ii) fields with no natural margins but with managed honeybee hives that had been placed in the beginning of the flowering season, resulting in few wild honeybees or other wild flower visitors, with managed honeybees being dominant (three fields). Fields with managed hives were selected at least 1 km from natural habitat, and fields with no managed hives were selected at least 1 km from managed hives. The maximum foraging distance of mass-marked honeybees was 955 m from their colonies (Osborne *et al*., 2001).

All fields selected had the same soil characteristics, but sunflower cultivar varied between fields (Pannar 7033, Pannar 7355, Syngenta and Monsanto DK4040). Thirteen plots (approximately 15m x 3 rows of flower heads each) were selected throughout the fields (see Table 2.1), seven plots near (30-230m) to the flower visitor source (either managed hives or natural habitat) and six plots far (815-1070 m) from the flower visitor source. All statistical analyses described hereafter were performed using Statistica 7.0 (1984-2004).

Pollination effectiveness of honeybees (managed and wild) and other flower visitors

Three different groups of flower visitors affecting pollination were distinguished: managed honeybees, wild honeybees, and other wild insects. To test pollination effectiveness of the different groups, in each plot, 45 sunflowers were selected (3 rows x 15 plants). Eighteen sunflowers (3 rows x 6 plants) were left open, receiving visits from all types of insects; nine sunflowers (3 rows x 3 plants) were bagged to exclude any insect visitation by placing a nylon mesh bag over the head, before any floret opened; the remaining eighteen sunflowers were also bagged (3 rows x 6 plants), but during the peak of flowering, the bag was removed to allow the visit from a single honeybee (Greenleaf and Kremen, 2006). If the focal bee was disturbed by other insects, that observation was discarded. As soon as the focal bee departed, the bag was replaced to exclude further insect visits. As some caterpillars emerged within bagged sunflower heads (Appendix), potentially influencing pollination, those heads were not used for the evaluation of pollination efficiency during single visits.

Sixty days later, when seeds had set and reached maturity, 100 seeds were randomly selected from each sunflower head in each site. The overall mass of the selected seeds was measured and used as a measure of seed set. These data was then used to answer the following questions:

Are wild flower visitors in general, and honeybees in particular, improving pollination?

The pollination efficiency of wild honeybees and other flower visitors as well as the distribution of sunflower visitors were evaluated in seven plots at different distances from natural habitat (four near vs. three far). In each plot, all flower heads were selected at the peak of flowering (i.e. around 50% of flowers are fully open), and flower visitors were observed for 4 minutes by a single observer. In each plot, visitation survey data was collected in the morning (09h00-13h00), and afternoon (14h00-17h00). Flower visitors that could not be identified in the field were collected, whenever possible, and kept in individual paper bags for later identification. The abundance data for flower visitors met the assumptions for parametric statistics; therefore the abundance of flower visitors was analyzed using main effects ANOVA with both distance from natural habitat (near vs. far) and cultivar as independent variables and number of honeybees and flower visitors as dependent variable.

To separate the contribution to sunflower pollination of honeybees from the contribution of other flower visitors, the seed set from sunflower heads that received no visits from insects (bagged), sunflower heads that were visited by a single honeybee, and sunflowers that were visited multiple times by multiple insects was compared. After log transformation, the data for seed mass met the assumptions for parametric statistics; therefore pollination efficiency was compared using main effects ANOVA with distance, cultivar and treatments (no visits *vs.* single visit *vs.* multiple visits) as categorical independent variables and seed mass as dependent variable. In addition, the effect of pollination for each sunflower cultivar was compared using one-way ANOVA separately using treatments (no visits *vs.* single visit *vs.* multiple visits) as categorical independent variable and seed mass as dependent variable. Only fields near to natural habitat were used and followed by post-hoc comparison to compare which groups differ from each other.

Are managed and wild honeybees equally efficient as pollinators?

To compare pollination effectiveness of managed and wild honeybees, the seed set from sunflower heads visited by a single honeybee from plots with managed honeybees (near managed honeybees and far from natural habitat) and with wild honeybees (plots far from managed honeybees and near natural habitat) was compared. The data for seed mass met the assumptions for parametric statistics; therefore pollination efficiency of managed and wild honeybees was compared using main effects ANOVA with honeybee source (managed honeybees vs. natural habitat) and cultivar as independent variables and seed mass as dependent variable.

Does the distance from natural fields affect the effectiveness of individual honeybees?

To compare the efficiency of wild honeybees at different distances from the honeybee source, only fields which had natural habitat as a source of flower visitors were used. The seed set from sunflower heads visited by a single honeybee from plots near to natural habitat and far from natural habitat was compared. After log transformation, the data for seed mass met the assumptions for parametric statistics; therefore pollination efficiency of honeybees at different distance from honeybee source was compared using main effects ANOVA with distance to natural habitat (near vs. far) and cultivar as independent variables and seed mass as dependent variable.

Foraging behaviour of honeybees

In each plot foraging behaviour surveys were done in the morning (09h00-13h00), and afternoon (14h00-17h00), the optimum foraging time for honeybees (Du Toit, 1988). All flower heads selected for observations were at the peak of flowering (i.e. around 50% of flowers were fully open). All foraging activities performed by the newly arrived individual honeybees, foraging alone on open flowers, were observed. Other insects (including honeybees) approaching the focal honeybee were prevented gently by the observer from landing on that flower to avoid disturbance. If the focal honeybee was disturbed either by other insects or the observer that observation was abandoned. The type of floral rewards (pollen vs. nectar) collected and activities performed on the disc florets of sunflower head were recorded. The following foraging activities of honeybees were recorded. i) whether honeybees were pollen dusted or not on their body hairs, ii) if honeybees discarding pollen from their body hairs and legs on flower head, or iii) if honeybees were packing pollen into their corbiculae iv) percentage of honeybees collecting nectar.

Foragers were subsequently categorized as nectar collectors (honeybees inserting their head into the corolla of a floret and with no pollen loads on their corbiculae), pollen collectors (honeybees with pollen loads on their corbiculae) or nectar and pollen collectors (honeybees collecting both nectar and pollen). In addition, the stages of the florets visited (male or female stage) were also noted. All the data were collected under warm temperatures (25 to 39^0C), mild wind (speed < 6m/s) and humidity levels between 32 and 73% RH. The type of resource collected was analyzed using a Chi-square test to compare if there is a difference in resource preference (nectar or pollen).

Results

Pollination effectiveness of honeybees (managed and wild) and other flower visitors

Are flower visitors in general, and honeybees in particular, improving pollination?

The most abundant sunflower visitors in fields with no managed honeybees were wild honeybees, representing 90.27% of the total number of visitors. Other flower visitors included Lepidoptera (2.7%), Coleoptera (2.16%), Diptera (2.16%), Heteroptera (1.62%), and other Hymenoptera (1.08%) (Table 2.2). Because of the low number of wild flower visitors other than honeybees, all these potential pollinators were pooled together for statistical analysis. The abundance of both honeybees and other flower visitors decreased slightly with distance from natural habitat (wild honeybees, near: 1.61 ± 0.32 honeybees/head, far: 1.21 ± 0.38 honeybees/head; other flower visitors, near: 0.17 ± 0.13 visitor/head, far: 0.15 ± 0.14 visitor/head). However this decline was not statistically significant (see Tables 2.3 and 2.4)

The overall sunflower seed yield (mass of 100 seeds) was significantly affected by distance from natural habitat, cultivar and treatments (no visit vs. single visit vs. multiple visits; Table 2.5). In separate analysis for each cultivar, there was a significant difference in mass of seeds between treatments (no visit, single visit and multiple visits) for Pannar 7033 ($F = 19.86$, $df = 2$, $P < 0.0001$) and Pannar 7355 ($F = 4.58$, $df = 2$, $P < 0.02$) but not for Monsanto DK 4040 ($F = 3.06$, $df = 2$, $P = 0.06$). The post-hoc analysis revealed that open pollinated flowers produced significantly heavier seeds than single honeybee visited and bagged flower heads in the two Pannar cultivars (Table 2.6).

Are managed and wild honeybees equally efficient as pollinators?

The overall model on the pollination effectiveness of managed and wild honeybees was highly significant ($R^2 = 0.50$, n = 70, $P < 0.0001$) with sunflower cultivar contributing to the model ($F = 18.97$, $df = 3$, P < 0.0001) but not honeybee source ($F = 2.38$, $df = 1$, $P = 0.128$).

Does the distance from natural fields affect the effectiveness of individual honeybees?

The overall model on the pollination effectiveness of individual honeybees was highly significant ($R^2 = 0.44$, n = 70 and $P < 0.0001$) and only sunflower cultivars contributed to the model ($df = 2$, $F = 16.191$, $P < 0.0001$) but not the distance from natural habitat ($df = 1$, $F = 3.425$, $P = 0.068$). However, there was a slight reduction in seed mass with distance from natural habitat for each of the cultivars (Pannar 7033: 3.74 g ± 0.84 vs. 3.42 g ± 1.07; Pannar 7355: 4.87 g ± 1.68 vs. 4.10 g ± 1.56 for near and far plots from natural habitat respectively). No comparison was possible for cultivar Monsanto DK 4040 since there was only a single plot near to natural habitat.

Foraging behaviour of honeybees

Significantly more foragers were collecting nectar as compared to pollen (Chi-square = 115.89, *df* = 1, *P* <0.0001). Of the total 132 honeybees observed when foraging alone on open sunflowers, 97 (73.5%) were collecting nectar, 11 (8.3%) pollen and 24 (18.2%) both nectar and pollen. Concerning the distribution of pollen the majority of nectar collectors (75 out of 97 individuals, 77.3%) were discarding pollen dust from their body hairs on the florets of sunflower heads. Honeybees visited different stages of florets of sunflowers to exploit the available floral rewards. Seventy-two out of 132 honeybees (54.5%) visited both female and male stages of florets in the same visit; while 55 (41.7%) visited only male stages and 5 (3.8 %) only female stages of florets of sunflowers.

Discussion

Pollination effectiveness of honeybees (managed and wild) and other flower visitors

Are flower visitors in general, and honeybees in particular, improving pollination?

Contrary to previous results (Steffan-Dewenter and Tscharntke, 1999; Chacoff and Aizen, 2006), the distribution of wild honeybees and other wild pollinators only slightly decreased with distance from natural habitat in this study. Areas with abundant natural flower resources can serve as a source of pollinators for neighbouring crops (Chacoff and Aizen, 2006). Possibly the presence of flowering plants (weeds) within the crop fields can help to minimize reductions of sunflower visitors by providing nests and floral rewards. However, the mass of seeds was significantly affected with distance from natural habitat. This shows that sunflower pollination service may be sensitive to changes in the abundance of wild pollinators.

Previous studies in different countries of the world have investigated the importance of bees to sunflower pollination (Butz Huryn, 1997; Kamler, 1997; Basualdo, 2000; De Paiva *et al*., 2003; Sumangala and Giriraj, 2003; Hernandez, 2008; Oz *et al*., 2009).

In our study, two self-fertile sunflower cultivars (Pannar 7033 and Pannar 7355) produced significantly heavier seeds when exposed to insect visitation. Although the increment was not statistically significant for the cultivar Monsanto DK 4040, also produced heavier seeds when exposed to multiple visits. A possible reason for the heavier sunflower seeds is that open pollinated flowers are more likely to receive sufficient viable pollen on the stigma from multiple visits by honeybees and other potential insect visitors. Moreover, the contribution of open pollination and single visits from wild honeybees varied among cultivars. In this study the highest increment of seed mass from open pollination (multiple visits in open pollinated sunflower heads) was recorded on cultivar Pannar 7033 (35.4%), followed by Pannar 7355 (27.6%) and Monsanto DK 4040 (14.1%) respectively. However, there were no significant differences in seed mass between single bee visited and insect visitation excluded sunflower heads in all the three cultivars. Our result is in agreement with other studies that showed that different sunflower cultivars responded to honeybee pollination differently (Low and Pistillo, 1986; Kamler, 1997; De Grandi-Hoffman and Chambers, 2006). For example, sunflower cultivars with a high level of autogamy increased seed yield by 35% and cultivars with a medium level of autogamy increased between 50% to 140% due to insect pollination especially honeybee pollination (Freund and Furgala, 1982). In caged hybrid sunflower pollination honeybees improved seed yield per head by 206% as compared with a caged pollination experiment without honeybees (Oz *et al*., 2009). Kamler (1997) reported that the quantity of seed yield increased from <1% up to 101% due to honeybee pollination, depending on cultivars. De Paiva *et al.* (2003) found honeybee pollination increased the production of sunflower seeds by 78 percent. Du Toit (1988) also reported an increment of 38% in sunflower seed production in cages with honeybees, compared to those without honeybees**.** In general, insect pollination, particularly honeybee pollination, is essential to maximize sunflower production.

As in a previous study on honeybee foraging behaviour (Monzon *et al*., 2004), our results show that honeybees came in contact with a larger number of stigmas (i.e. florets) per foraging trip on previously bagged flowers as compared to open pollinated flowers. Monzon *et al*. (2004) suggested that sometimes there could be overestimation of the pollination effectiveness of single visit experiments, since previously unvisited virgin flowers are expected to have a higher amount of pollen and nectar compared to open pollinated flowers. Furthermore, although the flower heads were bagged carefully, some florets could be rubbed by mesh bags across the top of the sunflower head and may have received pollen from adjacent florets. It was impossible to avoid contact completely. Therefore, it is likely that some of our values from single visit and insect exclusion experiments are overestimated, and hence the role of open flower visitation is underestimated. It is clear that the pollination service from wild flower visitors in general is essential to increase seed yield for all the studied sunflower cultivars. Although the efficiency of single honeybee visits is not clear, since honeybees constituted the large majority of flower visitors, the overall contribution of this species is likely to be larger than for any other flower visitor species.

Are managed and wild honeybees equally efficient as pollinators?

Our results showed no significant differences in seed mass between fields with managed honeybee colonies and those without managed honeybee colonies. Although information is lacking about the population size of wild honeybee colonies surrounding our sunflower fields, Moritz *et al*. (2007) found the density of wild honeybee colonies in three nature reserves of South Africa was significantly higher than in two national parks in Germany (range between 12.4 and 17.6 vs. 2.4 and 3.2 colonies per square km). During the study we also found wild honeybees nesting and producing honey on the branch of a tree within a natural habitat margin (Fig. 2.1). Moreover, several studies have mentioned the importance of wild honeybee populations for pollination of various plant species (Aizen and Feinsinger, 1994; Yates *et al*., 2005). This suggests that, when there is natural habitat surrounding sunflower fields, wild honeybee colonies provide as effective sunflower pollination service as managed honeybee colonies. Similarly, Radford *et al*. (1979) found that feral honeybee populations within 3 km from natural habitat are sufficient for sunflower pollination in Queensland, Australia. Other researchers also stated that feral honeybees are as important apple pollinators as managed honeybee colonies in Michigan (Chang and Hoopingarner, 1991), and the most abundant pollinators of *Lavandula* *latifolia* in Spain (Herrera, 1989). Thus, pollination service is positively correlated to natural habitat in the vicinity of agricultural farms (Kremen *et al*., 2004). Perennial plants flowering at different seasons of the year in the surroundings of agricultural fields may provide essential floral resources outside the crop flowering season, and are therefore important to pollinator conservation (Tuell *et al*., 2008). Thus for sunflower pollination in the Settlers area, pollination due to wild honeybees will be improved if fields border natural areas.

Does the distance from natural fields affect the effectiveness of individual honeybees?

The pollination efficiency of a flower visitor is defined as its contribution to reproductive success of a plant after a single visit (Larsson, 2005). Although our result shows that the mass of seeds from a single visit was not statistically affected by distance, there was a slight reduction in seed mass with distance from natural habitat. This suggests that pollination efficiency of individual wild honeybees may be correlated with the abundance of other pollinators (other species or simply other honeybees). This trend is in agreement with Greenleaf and Kremen (2006) who found that honeybee pollination produced a greater number of hybrid sunflower seeds per single visit with high abundance of wild bees. This is because the presence of wild bees increased the per visit effectiveness of honeybees in sunflower pollination.

Foraging activities of honeybees

This study revealed that the majority of honeybees are more attracted by nectar rewards than pollen rewards of sunflower. Similar foraging behaviour has been observed in previous studies (Tepedino and Parker, 1982; Fell, 1986; Skinner, 1987; Free, 1993; De Paiva *et al*., 2002). As reported by Free (1993) nectar collectors were highly dusted with pollen while pushing their tongue and head between the petals and anther tube to reach the base of the corolla (Fig. 2.2) and they discarded the pollen on the flower head. Honeybees collecting nectar deposited more pollen on stigmas, although they removed less pollen as compared to pollen collectors (Young *et al*., 2007). This foraging behaviour makes nectar collectors effective pollinators of sunflower (Free, 1993; De Paiva *et al.*, 2002). In addition, most of the bees visited male stages of sunflower florets. This result is in agreement with Fell (1986) who stated that for most of the day honeybees visited male stages of florets, although they visited female stages of florets in the early morning to collect residual nectar produced in the previous day. This is because of the greater amount of nectar that is produced by male stages of florets (McGregor, 1976). According to McGregor (1976) every morning at about 8h30 when male stages of florets started to dehisce, honeybees shifted from visiting female stages of florets to male stages of florets.

Small numbers of honeybees collected sunflower pollen, of which only a small proportion was discarded on sunflower heads while cleaning their body with their legs to accumulate the pollen in their corbiculae. As honeybees wet the pollen with nectar to load it more easily into their corbiculae (Young *et al*, 2007), pollen loads on their corbiculae are not suitable to be transferred on the stigmas (Michener, 1999). The reason why few honeybees were observed collecting sunflower pollen may be because sunflower pollen is not very attractive to honeybees (Free, 1964), and hence honeybees fulfilled their pollen requirements from other competing floral resources elsewhere (Tepedino and Parker, 1982). A study in southern Argentina showed that European honeybees are less attracted to sunflower flowers, and hence collected more pollen and nectar rewards from the flora near to the sunflower fields (Andrada *et al*, 2004). On the other hand, Basualdo *et al.* (2000) observed that Africanized honeybees would be the most efficient pollinators in commercial sunflower hybrid seed production. This is because Africanized honeybees collected greater proportions of sunflower pollen and were less attracted to flora competing with sunflower compared to European honeybees. However, among 41 taxa identified for pollen analysis in Turkey, sunflower was the second preferred floral resource by honeybees (Biliski *et al.*, 2008). Thus, the preference for sunflower pollen may vary according to the availability of other competing co-flowering plants and honeybee subspecies.

Conclusion

In general insects are essential to improve sunflower production. Although insect pollination, particularly honeybee pollination, improved the mass of seeds in all self-fertile sunflower cultivars in this study, each cultivar shows different level of response when exposed to insect visitation. Interestingly, wild honeybee colonies are found to be as efficient as managed honeybee colonies in sunflower pollination near to natural habitat. The majority of honeybees were collecting nectar and made a great contribution in transferring pollen dust from their bodies to the stigmas. This foraging behaviour makes nectar collectors important pollinators of sunflower. Sunflower yield is directly correlated with the abundance of pollinators. However, both sunflower yield (in terms of seed mass) and the abundance of pollinators decreased at 1 km from natural habitat, and larger distances may produce bigger differences. Therefore conservation of natural habitat as a source of wild honeybees and other wild pollinators is recommended near to sunflower fields, whereas fields larger than 1 km are not ideal for optimal pollination.

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Table 2.1. Summary of the study plots in Settlers commercial sunflower farms

Field	Plots	Cultivar	Flower visitor source	Number of managed hives	Distance from flower visitor source (m)
H3b	1	Pannar 7355	Natural habitat	0	30
JJ2	2	Pannar 7355	Natural habitat	0	50
WG2	3	Pannar 7033	Natural habitat	0	50
WD3	4	Syngenta	Managed hives	22	70
WW3	5	Monsanto DK 4040	Natural habitat	0	80
H3	6	Pannar 7355	Managed hives	15	100
E1	7	Pannar 7033	Managed hives	43	230
JJ2	8	Pannar 7355	Natural habitat	0	815
WD3	9	Syngenta	Managed hives	22	970
H3b	10	Pannar 7355	Natural habitat	0	980
H3	11	Pannar 7355	Managed hives	15	1000
WG2	12	Pannar 7033	Natural habitat	0	1000
E1	13	Pannar 7033	Managed hives	43	1070

Table 2.2. Sunflower visitors in Settlers commercial fields near to natural habitat

Order	Family	Species
Coleoptera	4 morphospecies	
Diptera	Sarcophagidae	*Sarcophaga* sp.
Diptera	Syrphidae	*Betasyrphus adligatus (*Wiendemann)
Diptera	Syrphidae	*Senaspis haemorrhoa (*Gerstaecker)
Hymenoptera	Apidae	*Tetraloniella apicalis* (Friese)
Hymenoptera	Apidae	*Xylocopa caffra* (Linnaeus)
Hymenoptera	Halictidae	*Lasioglossum* sp.
Hymenoptera	Megachilidae	*Megachile frontalis (*Smith*)*
Hymenoptera	Scoliidae	*Campsomeris* sp.
Heteroptera	3 morphospecies	
Lepidoptera	Arctiidae	*Utetheisa pulchella* (Linnaeus)
Lepidoptera	Hepialidae	*Eudalaca exul* (Herrich-Schäffer)
Lepidoptera	Nymphalidae	*Junonia oenone* (Linnaeus)
Lepidoptera	Nymphalidae	*Hypolimnas misippus(*Linnaeus)
Lepidoptera	Nymphalidae	*Cynthia cardui* (Linnaeus)
Lepidoptera	Nymphalidae	*Danaus chrysippus* (Linnaeus)
Lepidoptera	Sphingidae	*Macroglossum trochilus* (Hubner)

Table 2.3. Results of main effects ANOVA comparing the effects of distance and cultivar on the distribution of wild honeybees on sunflowers in Settlers commercial farms. Overall statistics: Adjusted $R^2 = -0.01$, n = 115 sunflower heads and $P = 0.508$.

Factors	SS	*df*	*F*	*P*
Distance	0.177	1	1.226	0.349
Cultivar	0.150	2	0.517	0.641

Table 2.4. Results of main effects ANOVA comparing the effect of distance and cultivar on the distribution of wild flower visitors on sunflowers in Settlers commercial farms. Overall statistics: Adjusted $R^2 = -0.12$, n = 115 and $P = 0.576$

Factors	SS	d.f.	*F*	*P*
Distance	0.007	1	0.431	0.558
Cultivar	0.038	2	1.173	0.420

Table 2.5. Results of main effects ANOVA comparing the effects of distance, cultivar and treatments (no visit vs. single visit vs. multiple visits) on the mass of 100 seeds of sunflowers in Settlers commercial farms. Overall statistics: Adjusted $R^2 = 0.46$, n = 202, and $P < 0.01$

Factors	SS	*df*	*F*	*P*
Distance	57.568	1	27.321	<0.0001
Cultivar	202.806	2	48.125	<0.0001
Treatment	97.274	2	23.083	<0.0001

Table 2.6. Results of post-hoc analysis (Tukey HSD test) comparing the effect of treatments (bagged vs. single visit vs. open pollinated) on the mass of sunflower seeds. Values in the table are mean mass of 100 seeds (g) ± standard deviations (SD) of the three sunflower cultivars.

	Sunflower cultivars		
Treatments	Pannar 7033	Pannar 7355	Monsanto DK 4040
Bagged	4.47^{a**} ±1.19 (n = 10)	4.36^{a*} ± 1.48 (n =18)	7.62^{a} ± 1.36 (n = 9)
Single bee visited	3.74^{a} **± 0.84 (n = 9)	4.87^{a} ± 1.68 (n = 22)	7.71^{a} ± 1.18 (n = 12)
Open pollinated	6.92^{b}± 1.17 (n = 8)	6.02^{b} ± 1.98 (n = 21)	8.87^{a} ± 1.12 (n = 9)

Different letters in each column indicate significant differences between treatment means.

* $P < 0.02$; ** $P < 0.001$

Fig. 2.1. Wild honeybee colony nesting on the branches of acacia tree in natural habitat near to a commercial sunflower field in Settlers.

Fig. 2.2. Nectar collecting honeybee highly dusted with sunflower pollen.

CHAPTER 3

Sunflower pollination benefits from the behavioural interactions of honeybees with wild pollinators

Introduction

Insect visitation increases cross pollination of several crop species (e.g. Greenleaf and Kremen, 2006; Karron *et al*., 2009), potentially leading to better seed/fruit production (Donaldson *et al*., 2002; DeGrandi-Hoffman and Chambers, 2006; Albrecht *et al*., 2007). Although self-fertilization can occur in some sunflower cultivars, pollen from different flower heads has a higher fertilization rate than self pollen (Joksimovic *et al*., 2005), leading to higher seed production (Free, 1993). Honeybees are the main managed pollinators used for sunflower pollination worldwide, increasing the quality and quantity of seed yields (Free, 1964; Langridge and Goodman, 1974; Radford *et al*., 1979; El-Sarrag *et al*., 1993; De Paiva *et al*., 2003; De Grandi-Hoffman and Chambers, 2006; Nicoleta *et al*, 2007; Oz *et al*., 2009). Several other insects also visit sunflowers (El-Sarrag *et al*., 1993; Nicoleta *et al*, 2007). Although wild bees can be as efficient as honeybees in sunflower pollination (Greenleaf and Kremen, 2006), due to their low abundance their direct contribution to sunflower pollination was thought to be insignificant (Radford *et al.,* 1979). The term wild bee is defined as all bees except honeybees in the genus *Apis*. However, recent studies show that wild bees have an important indirect contribution for sunflower pollination, by making honeybees collect more pollen per visit and move between male and female rows of sunflowers more frequently (Greenleaf and Kremen, 2006). Further studies are needed to clarify the details of these behavioural interactions between honeybees and other wild pollinators, and the consequent contribution to sunflower pollination.

Pollination effectiveness can be improved by a reduction in the duration of visits to hybrid sunflowers (Greenleaf and Kremen 2006). Two factors can influence visit duration: i) availability of floral rewards (pollen and nectar), where a larger amount of available resources leads to a longer foraging visit (Neff and Simpson, 1990; Manetas and Petropoulou, 2000) and ii) competition among individuals (inter- and intraspecific competition), with an overlap in exploitation of floral rewards from the same plant species leading to more frequent movement (Paini, 2004; Yoshimoto and Nishida, 2009). Moreover, as the species richness and abundance of insects observed in an area is influenced by the abundance of food resources (Yoshimoto *et al*., 2005), exploitative competition pressures are likely to increase with food resource availability.

The abundance of wild bees can decline in the presence of both managed and wild honeybee colonies due to competition for nectar and pollen (Kato *et al*., 1999). Furthermore, Martins (2004) observed that honeybees can displace wasp and *Precis hierta* butterflies from *Acacia senegal* (acacia-commiphora) and wild bees from aloes on many occasions. In addition, managed honeybees can competitively suppress the foraging behaviour of bumble bees, *Bombus occidentalis* (Thomson, 2004). However, Yates *et al*. (2005) found that wild honeybees did not have an impact on wild pollinators in the case of availability of unlimited floral resources. In addition, some wild bees can successfully compete with honeybees (Donovan, 1980). Similarly, Steffan-Dewenter and Tscharntke (2000) reported that species richness and abundance of wild bees is not affected by the density of honeybee colonies in resource competition to exploit floral rewards. Moreover, Aizen and Feinsinger (1994) found that the visitation frequency of wild honeybees to flowers was lower where the species richness and visitation frequency of native flower visitors was higher. In addition, Fell (1986) investigated that bumble bees displaced honeybees from more attractive sunflower oilseed sunflower cultivar and forced to visit less attractive cultivar. Therefore, honeybee foraging behaviour may be affected by interspecific competition with different species of flower visitors. Interspecific competition occurs when individuals of different species forage for a limited resource, either for food resources or territories when one species reduces the access of another species (Takeuchi, 2006; Yoshimoto and Nishida, 2009), leading to important behavioural changes (e.g. Cresswell, 1997; Yoshimoto and Nishida 2009), and potentially increasing the movement of foraging species (Primack and Howe, 1975; Galindo-González and Ornelas, 2002).

Although there are several studies on behavioural interactions between honeybees and other flower visitors, honeybee responses are generally variable. For example, honeybees were found to be the most dominant visitors in highly productive habitats (Schaffer *et al*., 1979; Butz Huryn, 1997), while in a feeding experiment the number of honeybees declined due to fighting with wasps when the food resource was limited (Markwell *et al*., 1993). The reasons behind these different responses to flower visitor community changes are still not clear. The aim of this study was i) to investigate the impacts of food resource availability on visitation length and number of florets visited per min for nectar and/or pollen on a single sunflower head, as well as ii) to investigate the influence of inter- and intraspecific competition on honeybee forager movement as well as nectar and pollen collection.

Materials and methods

Study areas

Settlers sunflower farms

The effect of resource availability on visitation length and the number of florets visited per minute for nectar/or pollen on a single sunflower head as well as the influence of inter- and intraspecific competition on honeybee forager movement and foraging time per flower head was observed in Settlers commercial sunflower fields located in Limpopo Province, South Africa (27^0 57’S, $28^0$32’E) during the 2009 sunflower flowering season (17 March - 08 April).

Experimental farm

Observations on the properties of honeybee collected nectar and competition during foraging were made on the experimental farm of the University of Pretoria (25^0 45’S, 28^0 16’E) during the 2009 sunflower flowering season (6 May - 14 May). The volume, sugar amount and sugar concentration of nectar collected from bagged and open pollinated flowers, as well as from the crops of honeybees foraging on caged and open pollinated flowers, were compared. In addition, the effect of inter- and intraspecific competition on honeybee forager movement and foraging time per flower head as well as on nectar and pollen collection was observed.

Effect of resource availability on honeybee foraging behaviour

To test if an increase in resource availability would affect honeybee foraging behaviour, 80 sunflower heads were randomly selected in the experimental farm, 40 of which were bagged with fine mesh bag before flowering and the remaining 40 were left open. Seven days later, when sunflower heads were at the peak of flowering, the bag was removed, and the nectar from 15 florets of bagged and unbagged sunflowers was collected and pooled using 2 µl or 1 µl capillary tubes (Broomall, Pennsylvania Drummond Scientific Company, USA). All samples were collected between 13h00 and 17h00 on 13 and 14 June 2009. Nectar volume was calculated from the column length in the capillary tubes (Human and Nicolson, 2008). The sugar concentration of nectar was measured using a hand held refractometer (40-85%, Bellingham and Standley Ltd, Tunbridge Wells, UK). The amount of sugar in nectar was calculated using the following formula (Dafni, 1992; Human and Nicolson, 2008).

$$\text{Amount of sugar per 15 florets (mg)} = \frac{\text{nectar volume } (\mu\text{l}) \text{ x nectar sugar concentration } (\%) \text{ x nectar density}}{100}$$

To determine if an increase in resource availability would affect the foraging rate of honeybees, i.e. the number of florets visited per min on a single sunflower head, 103 sunflower heads were randomly selected in the Settlers commercial farms, 48 of which were bagged with fine mesh bag before flowering and the remaining 55 were left open. When sunflower heads were at the peak of flowering the bag was removed. Subsequently, the foraging rate of individual honeybees was recorded when visiting on previously non-visited (bagged) and visited (open) sunflower heads.

To evaluate if an increase in resource availability would affect the foraging time spent by individual honeybee per flower head (visitation length), 276 sunflower heads were randomly selected in the Settlers commercial farms, 144 of which were bagged with fine mesh bag before flowering and the remaining 132 were left open. When sunflower heads were at the peak of flowering the bag was removed. Afterwards, the visitation length of individual honeybees was recorded when visiting all previously non-visited (bagged) and visited (open) sunflower heads.

The Mann-Whitney *U* test was used to compare the volume, sugar concentration and sugar amount of the nectar collected from bagged and open sunflowers as well as to compare honeybee visitation length on bagged flowers and previously visited flowers (the data were not normally distributed). The foraging rate data on the number of florets visited per min in bagged and previously visited flowers met assumptions for parametric statistics and groups were compared using the student's t-test.

Effect of inter- and intraspecific interactions in honeybee foraging behaviour – forager movement

Behavioural interactions of honeybees with wild pollinators were observed on 16 commercial sunflower farms in Settlers, situated at different distances from natural habitat (30-1900 m), and also at the experimental farm, situated at about 400 m from natural habitat. Flower heads selected for observations were at the peak of flowering (i.e. around 50% of the florets were fully open). A total of 320 individual honeybees were observed foraging alone on sunflowers until the focal honeybee interacted with another honeybee (n =139), a butterfly (n = 91), a wild bee (n = 60) or a moth (n = 30) respectively. Forager movement (movement of the pollinators from flower to flower) was recorded. The time spent (in minutes) by honeybees on the sunflower head while interacting with wild pollinators was also recorded and the focal honeybee was followed to determine whether it remained on the same flower head or moved to another flower head after the interaction (see also Greenleaf and Kremen, 2006).

Due to the difficulty of identifying insects in the field, insects were classified into four categories: honeybees, wild bees, butterflies and moths. When identification to species level was not possible on site, individuals were collected after the observation, when possible, for later identification.

Fisher's exact test was used to determine the effect of wild bees, butterflies and moths on the movement of honeybees from one flower head to another when interacting with honeybees. The number of movements versus non-movements was compared for each pollinator group separately. Data on honeybee foraging time was not normally distributed, so statistical comparisons were made using Kruskal-Wallis ANOVA, testing the overall effect of all interactions on foraging time of honeybees, and the Mann-Whitney U test for comparison of two independent samples.

Effect of inter- and intraspecific interactions in honeybee foraging behaviour - nectar and pollen collection

To investigate whether honeybees collect more or less resources when competing with other pollinators, the pollen loads and crop nectar content (hereafter crop loads) of honeybees in the presence and absence of other pollinators were measured on a sunflower field located at the experimental farm. Six plots (5 m long x 3 rows each) were selected near to natural habitat (about 400 m) to increase the chance of getting wild pollinators, and three of them were covered with insect proof mesh cages (each 5 m long x 3 m wide x 2.5 m high; 2 mm mesh size; Fig.3.1) before the ray florets started opening, to exclude pollinators. In the beginning of flowering (2 May 2009), one honeybee hive (four frames) was placed in each of the three sunflower plots inside mesh cages (Fig. 3.1).

To measure pollen loads of the individual bees, pollen samples were collected from honeybees foraging between 09h00 and 12h00 on 7 and 9 June 2009. A total of 70 honeybees foraging on flowers inside the mesh cage and 69 honeybees foraging on sunflower heads outside the mesh cage, all with pollen on their corbiculae, were used. When a honeybee finishes foraging, it flies several centimeters upwards before flying off (this behaviour helps to determine when the honeybee is ready to return to its hive with a full pollen load; Raw, 2000). Honeybees showing this behaviour were captured and kept individually in small plastic bottles, which were placed in freezer. Pollen load was later scraped off the hind legs of each individual using a dissecting pin, then dried at 45^0C for 24 h and weighed using an electronic balance with 0.1 mg precision (AG64, microsep (pty) ltd, Switzerland).

The behaviour of individual honeybees was observed for 3 min on sunflower heads and then 46 nectar collectors (no pollen on the legs) were sampled from both the covered and open plots between 13h00 and 17h00 on 7 and 9 June 2009. The crop nectar content of the selected foragers was measured by squeezing bees to regurgitate their crop load into a 75 mm capillary tube, capacity 75 µl (Kajobe, 2007; Nicolson and Human, 2008). The sugar concentration of nectar was measured to the nectar collected from sunflower heads. Furthermore, the sugar concentration of nectar collected from open pollinated sunflower heads and honeybee crop loads while foraging on open plot flowers were compared. Data for pollen loads from honeybee corbiculae, sugar concentration and sugar amount of the nectar collected from honeybees foraging on open plots and caged flowers met the assumptions of parametric statistics. Hence statistical analysis was done using student's t-test. The Mann-Whitney *U* test was used to compare the crop nectar volume of bees visiting sunflower florets in open and caged plots as well as the sugar concentration of nectar from honeybee crops and sunflower florets (data were not normally distributed).

Results

Effect of resource availability on honeybee foraging behaviour

The volume of nectar collected from bagged flowers (median, 0.72 µl/15florets; range, 0.38-1.88 µl/15 florets) was significantly higher than the nectar volume of previously visited flowers (median, 0.13 µl/15 florets; range, 0.06-0.31 µl/15 florets (Table 3.1). Moreover, the sugar content of nectar taken from bagged flowers (median, 0.56 mg; range, 0.28-1.21 mg) was significantly higher than that of unbagged flowers (median, 0.09 mg; range, 0.05-0.27 mg). However, there was no significant difference in nectar sugar concentration between open pollinated flowers (median, 62% w/w; range, 58-66% w/w) and bagged flowers (median, 61.5% w/w; range, 43-70.5% w/w) (Table 3.1).

The number of florets visited by a single honeybee per min on open flower heads was significantly higher than on non-visited flower heads (open flowers: 13.0 ± 4.48 florets per min, n = 48, bagged flowers: 7.58 ± 3.03 florets per min, n = 55; *t*-test = -7.08; $P < 0.0001$, Fig.3.2).

The time spent by honeybees foraging on previously non-visited (bagged) flowers (median, 5.99 min per sunflower head; range, 0.02-18.85 min per sunflower head; n = 144) was significantly higher than from honeybees visiting open flowers (median, 1.98 min per sunflower head; range, 0.1-22.32 min per sunflower head; n = 132) (Mann-Whitney *U* test: $z = 5.34$; $P < 0.0001$, Fig. 3.2).

Effect of inter and intraspecific interactions in honeybee foraging behaviour – forager movement

Several insect groups interacted with honeybees while foraging on sunflowers; among these butterflies, wild bees and moths were observed in terms of their behavioural interaction with honeybees (Table 3.2).

The frequency of movement of honeybees from flower to flower was significantly higher when they interacted with wild bees (Fisher's exact test, n = 60, $P < 0.002$, 73%), butterflies (Fisher's exact test, n = 91, $P < 0.0001$, 94.5%) and moths (Fisher's exact test, n = 30, $P < 0.0001$; 93.3%) than when they interacted with other honeybees (n = 139, 46%).

The overall honeybee foraging time per sunflower head when interacting with other pollinators was significantly lower than interaction among honeybees (Kruskal-Wallis ANOVA by ranks test: $H_{3,\ 320} = 29.971$; $P < 0.0001$). Furthermore, group comparison was performed using multiple comparisons (Table 3.3).

Effect of inter- and intraspecific interactions in honeybee foraging behaviour - nectar and pollen collection

There was no significant difference in the crop nectar volume of honeybees between open plots (median, 7 µl; range, 1.5-17 µl) and caged plots (median, 6.25 µl; range, 2 -12 µl). The crop nectar sugar concentration of honeybees collected inside the cages was significantly higher than for open plots (Table 3.4), but the sugar amount of honeybee crop nectar was not significantly different between open and caged plots (open: median, 4.19 mg; range, 0.79 - 12.05 mg; bagged: median, 4.25 mg; range, 1.26-8.31 mg) (Table 3.4).

The concentration of nectar collected from sunflower florets in open flowers was significantly higher than nectar collected from the crop of honeybee foraging on sunflowers in open plots (62.12% w/w ± 2.54, n = 40 vs. 48.0% w/w ± 4.39, n = 46; Mann-Whitney *U* test: $z = 7.94$; $P < 0.0001$). The average pollen loads collected from corbiculae of honeybees foraging in open plots were significantly higher than from those foraging in caged plots (Table 3.4).

Discussion

Effect of resource availability on honeybee foraging behaviour

Sunflower heads that had been isolated from flower visitors had higher sugar amounts compared to visited flowers. The concentration of sunflower nectar can range from 44.8 to 61.3% depending on air temperature and relative humidity in Hungary (Zajácz et al., 2006). In our study, an average concentration of nectar from open pollinated and bagged sunflower heads was 62.1% and 60.2% respectively. The possible interpretation for the slight difference in sugar concentration among treatments could be that bags on sunflower heads may have contributed to higher humidity compared to open flowers. Abiotic factors can affect the sugar concentration (Zajácz *et al*., 2006), causing it to vary throughout the day (Neff and Simpson 1990). For example, nectar concentration is positively correlated with temperature but inversely with relative humidity (Zajácz *et al*., 2006). Higher air temperature results in the higher sugar concentration of sunflower nectar through evaporation, but higher average humidity results in more diluted sunflower nectar due to moisture absorption (Zajácz *et al*., 2008).

This study revealed that the amount and quality (i.e. volume and sugar concentration) of nectar present in the florets of sunflower affects the visitation intensity of pollinators. These results are in agreement with previous studies (Neff and Simpson, 1990; Fulop and Menzel, 2000) which show that the behaviour of honeybees is influenced by the amount of rewards offered, honeybees spending more time and visiting fewer florets per min in bagged flower heads as compared to open pollinated flower heads. However, honeybees visited more florets per flower head per visit in bagged flowers compared to open pollinated flowers. This is because honeybees foraging on bagged flowers foraged almost twice as long compared to open pollinated flowers for a single trip. The results are likely related to higher accumulated amount of nectar in bagged sunflower heads. This implies that honeybees prefer to exploit floral rewards from the same source if they find the higher amount. This minimizes energy wastage by eliminating the need to fly and search for resources from neighbouring plants. Therefore, higher rewarding flowers will lead to less honeybee foraging movement, likely reducing cross-pollination

Effect of inter and intraspecific interactions in honeybee foraging behaviour - forager movement

In the current study, there were two major sources of competing insects: managed honeybee colonies transported to commercial sunflower fields during the flowering period and wild pollinators from natural habitat (including wild honeybees) surrounding the sunflower fields. Wild pollinators, other than wild honeybees, competed with honeybees (managed and wild) for nectar and pollen rewards of sunflower. Although intraspecific interactions also affected honeybee movement to a certain extent, interspecific interactions with other potential sunflower pollinators such as wild bees, butterflies and moths increased the movement of honeybees among sunflower plants. This result suggests that, in agreement with the findings of Greenleaf and Kremen (2006) for hybrid sunflower pollination in California, the pollination service of honeybees can be increased by increasing the probability of honeybees interacting with other pollinators. Therefore, conservation of natural habitat is essential as a source of nesting and floral resources for wild pollinators. Sunflower reproductive success is correlated with the abundance and richness of wild bees (Greenleaf and Kremen, 2006).

Butterflies were the most influential insects in enhancing honeybee foraging movements, followed by wild bees and then moths. The importance of a given flower visitor species to honeybee movement is likely related to the size of the visitor, as the bigger size of butterflies and movement of their wings when landing and sucking nectar on sunflower head. Consequently, honeybees foraging on the same flower were forced to leave the flower within a short period of time. Our results support the finding that wild bees contribute to make honeybees work faster on sunflower heads (Greenleaf and Kremen, 2006), though honeybees spent relatively longer time on flower head compared to interspecific interaction with butterflies. In addition, moths also obliged honeybees to move faster among sunflower plants than honeybees foraging with other honeybees.

Effect of inter- and intraspecific interactions in honeybee foraging behaviour - nectar and pollen collection

Honeybees visiting sunflower heads in open plots worked faster and collected significantly large pollen loads as compared to caged bees. There are two possible reasons for this effect. Firstly, interspecific competition with other pollinators made honeybees exploit more food resources. Greenleaf and Kremen (2006) also reported that honeybees interacting with wild bees carried more pollen on their bodies. Secondly, due to the confined environment inside a covered area of sunflower crop, honeybee foraging behaviour may be adversely affected.

According to Nicolson and Human (2008), the crop content of honeybees captured on flowers of *Aloe greatheadi var. davyana* was significantly more concentrated than the nectar sampled from flowers. This is because the more dilute nectar from the flowers is regurgitated onto the tongue and evaporated during foraging. In this study, therefore, it was hypothesized that the nectar sample from sunflower head would be more dilute than the crop contents of nectar foragers. However, honeybee crop nectar concentrations were lower than the nectar concentration of the florets (collected from open pollinated plots outside the cage 48.0% w/w ± 4.39 vs. 62.12% w/w ± 2.54). Similar result was reported by Neff and Simpson (1990), who found a lower crop nectar concentration compared to sunflower nectar concentration (17.8% vs. 18%) in the morning and (38% vs. 55%) in the afternoon. When given the choice honeybees prefer to collect nectar with a sugar concentration of between 30 and 50% (Waller, 1972), avoiding too low or too high nectar concentrations. This implies that honeybees avoid unnecessary energy consumption either to evaporate the water from low concentrated nectar or to dilute too concentrated nectar with their saliva. However, if no alternative resources are found honeybees will dilute highly concentrated sunflower nectar to their optimum concentration level, this being the most likely explanation for the differences in sugar concentration here reported. Another explanation for diluted crop content of honeybees could be honeybees drank water before they started nectar collection.

Conclusion

The availability of higher floral rewards limited honeybee movement between different sunflower plants, potentially having a negative effect on cross pollination. However, exploitative interspecific competition with wild pollinators made honeybees collect floral rewards more rapidly, with a simultaneous increase in their movement between flowers, and an increase in the number of florets touched on the same flower head. Thus, conservation of natural habitat is important to maintain the diversity of flower visitors, which have a great indirect contribution to crop production by enhancing honeybee foraging activity and consequent direct pollination service.

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Table 3.1. Results of the Mann-Whitney *U* test comparing the nectar of sunflower florets on open pollinated and bagged sunflower heads at the experimental farm. Sample size was 40 in all cases. Values in the table are mean ± SD nectar volume per 15 florets (µl), sugar concentration (%) and Sugar amount per 15 florets (mg) measured from open and bagged sunflower heads.

	Treatments			
Variables	Open pollinated flowers	Bagged flowers	*z* values	*P* values
Nectar volume/15 florets (µl)	0.13 ± 0.07	0.82 ± 0.35	-7.72	<0.0001
Sugar concentration (%)	62.12 ± 2.54	60.20 ± 6.85	0.89	0.37
Sugar amount (mg)/15 florets	0.11 ± 0.06	0.62 ± 0.21	-7.698	<0.0001

Table 3.2. Sunflower visitors interacting with honeybees for floral rewards on commercial sunflower fields at Settlers and the experimental farm of the University of Pretoria

Order	Family	Species	Pollinator	Sites
Hymenoptera	Apidae	*Tetraloniella apicalis*	Wild bee	Settlers
Hymenoptera	Apidae	*Allodapula variegata* (Smith)	Wild bee	Pretoria
Hymenoptera	Apidae	*Xylocopa caffra* (Linnaeus)	Wild bee	Pretoria
Hymenoptera	Apidae	*Thyreus calceata* (Vachal)	Wild bee	Pretoria
Hymenoptera	Halictidae	*Lasioglossum* sp.	Wild bee	Settlers and Pretoria
Hymenoptera	Halictidae	*Patellapis (Zonalictus)* sp.	Wild bee	Pretoria
Hymenoptera	Halictidae	*Allodapula variegata* (Smith)	Wild bee	Pretoria
Hymenoptera	Halictidae	*Saladonia* sp.	Wild bee	Pretoria
Hymenoptera	Megachilidae	*Megachile frontalis* (Smith)	Wild bee	Settlers
Hymenoptera	Megachilidae	*Afranthidium? junodi* (Friese)	Wild bee	Pretoria
Lepidoptera	Arctiidae	*Utetheisa pulchella*	Moth	Settlers
Lepidoptera	Hepialidae	*Eudalaca exul*	Moth	Settlers
Lepidoptera	Nymphalidae	*Junonia oenone*	Butterfly	Settlers
Lepidoptera	Nymphalidae	*Hypolimnas misippus*	Butterfly	Settlers
Lepidoptera	Nymphalidae	*Cynthia cardui*	Butterfly	Settlers
Lepidoptera	Nymphalidae	*Danaus chrysippus*	Butterfly	Settlers and Pretoria
Lepidoptera	Pieridae	*Catopsilia florella*	Butterfly	Pretoria
Lepidoptera	Pieridae	*Belonois aurota*	Butterfly	Pretoria
Lepidoptera	Sphingidae	*Macroglossum trochilus*	Moth	Settlers

Table 3.3. Results of post-hoc analysis (Tukey HSD test) on the effect of inter- and intraspecific interactions on honeybee foraging time spent per flower head.

Interaction	Duration per flower head (mean ± SD, in minute)	Number of observations
Honeybee with wild bee	1.49*^{a} ± 1.55	60
Honeybee with butterfly	0.95^{b} ± 1.17	91
Honeybee with moth	1.81** ^{a} ± 1.54	30
Honeybee with honeybee	1.89*** ^{a} ± 1.95	139

$^{a\ b}$ Different letters indicate significant difference between means.

* $P < 0.02$, ** $P< 0.004$, *** $P < 0.0001$

Table 3.4. Comparisons of the crop nectar content and pollen loads of honeybees collected from open pollinated and mesh caged sunflower heads (cultivar Monsanto DK 4040) at the experimental farm of the University of Pretoria. Data are mean ± SD. Sample size of all groups 46, unless indicated otherwise. Values in the table are crop volume (µl), sugar concentration (%) and Sugar amount (mg) collected from honeybee crop content foraging on open and caged sunflower heads.

	Treatments			
Variables	Open pollinated flowers	Caged flowers	*z/t* values	*P* values
Crop volume (µl)	7.45 ± 3.46	6.37 ± 2.41	1.13	0.25
Sugar concentration (%)	48 ± 4.39	52.89 ± 3.58	-5.85	<0.0001
Sugar content (mg)	4.43 ± 2.29	4.19 ± 1.64	0.00	1
Pollen loads (mg)	9.53 ± 3.27 (n = 69)	5.33 ± 2.06 (n = 70)	9.07	<0.0001

Fig.3.1. Insect proof mesh cage at the experimental farm of the University of Pretoria.

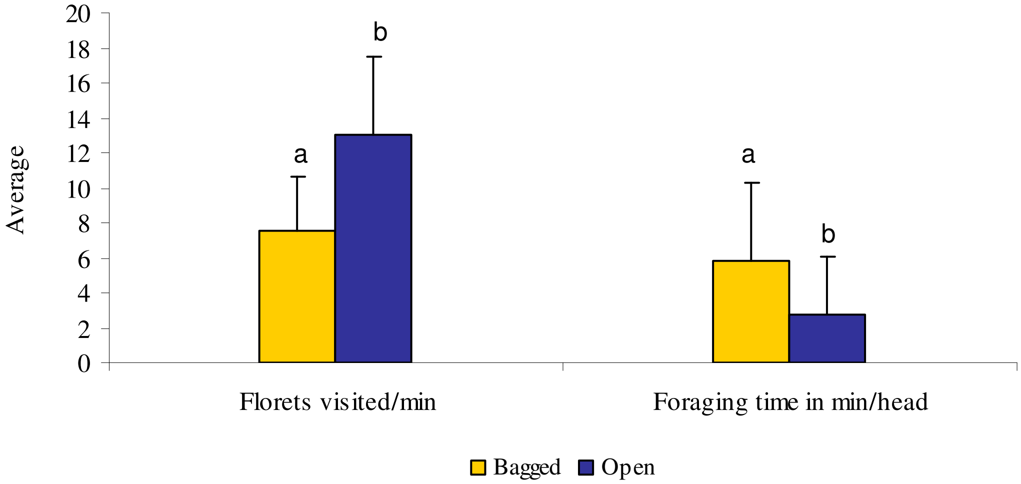

Fig. 3.2. Number of florets visited per min and time spent per flower on honeybees (*Apis mellifera scutellata* Lep.) foraging on previously bagged and open pollinated sunflower heads at Settlers commercial farms. Data are means ± SD (n = 276 for foraging time, n = 103 for foraging rate). Bars with different letters (a, b) are significantly different (*t*-test for florets visited/min and Mann-Whitney *U* test for foraging time in min/head; significance level $P < 0.0001$.

CHAPTER 4

General discussion

Improving our understanding of the factors that influence honeybee (managed and wild) foraging behaviour and pollination efficiency can contribute to the improvement of management practices that aim to enhance crop pollination. In this thesis, the importance of managed honeybee colonies and wild honeybees to sunflower pollination, the influence of sunflower nectar and pollen availability on the foraging behaviour of honeybees and the effects of exploitative interspecific competition on honeybee foraging behaviour were investigated. While comprehensive explanations are given in the preceding chapters, it is helpful to summarize and discuss the most important findings of this thesis. In addition, specific recommendations for sustainable sunflower pollination service are given and aspects to be examined in future research are suggested.

Sunflower pollination

The present study revealed that the most abundant sunflower visitors in fields with no managed honeybees were wild honeybees, representing 90.27% of the total number of sunflower visitors. Similar to previous results (De Grandi-Hoffman and Chambers, 2006; Nderitu *et al*., 2008), the present study showed that insect pollination in general, and pollination by honeybees (managed and wild) in particular, improved the mass of sunflower seeds in several self-fertile cultivars. As sunflower self-fertility varies among cultivars (Low and Pistillo, 1986), each cultivar shows a different level of response when exposed to insect visitation (Chapter 2).

The results of this study showed that there was no significant difference in pollination effectiveness between individual honeybees from managed hives and wild honeybees from natural habitat. Assuming that natural habitat is as good a source of honeybees as managed hives, sunflower fields near to natural habitat received sufficient pollination service from wild honeybee colonies and the other wild pollinator community. Furthermore, the distribution of wild honeybees and other wild pollinators only slightly decreased with distance from natural habitat. Possibly the presence of abundant flowering plants (weeds) within the crop fields (personal observation) helped to minimize reductions of sunflower visitors with distance. Previous research in Australia also found that the use of managed honeybee colonies is not important within 3 km of natural habitat (Radford *et al*., 1979). However, the production of sunflower was significantly reduced with distance from natural habitat. This could be in relation to slight reduction in pollinator abundance with distance from natural habitat.

Moreover, this study revealed that nectar collecting honeybees have the potential to contribute a greater proportion of sunflower pollination. Our result is in agreement with previous studies which stated that the majority of honeybees were collecting nectar, highly dusted with pollen while pushing their tongue between petal and anther tubes (Free, 1993), and deposited more pollen on the stigma (Young *et al*., 2007).

Honeybee response to floral rewards

Our results show that honeybee foraging behaviour was influenced by the amount of rewards offered. For example, with availability of higher amounts of floral rewards honeybees spent more time foraging and visited a higher number of florets per flower head (Chapter 3). This implies that honeybees prefer to exploit floral rewards from the same source if they find the higher amount, minimizing energy wastage for flight in searching for other sources from neighbour plants and this may have a negative impact on cross-pollination.

Advantages of interspecific interactions

The results from the field experiment (Chapter 3) reveal that interspecific competition was beneficial for sunflower pollination in two ways. First, it increased the movement of honeybees between sunflower plants, while reducing the length of foraging time spent by individual honeybees per sunflower head. The importance of a given flower visitor species to honeybee movement is likely related to the size of the visitor, butterflies (larger and with conspicuous movement of their wings) being the most influential in enhancing honeybee foraging movement, followed by wild bees and then moths. Second, the amount of pollen resource collected by honeybees when competing with wild pollinators was significantly higher compared to honeybees foraging in caged flowers. This result concurs with previous research that found that interspecific interaction causes honeybees to collect more pollen per single visit trip (Greenleaf and Kremen, 2006).

Although some studies have examined exploitative competition between honeybees and wild pollinators and concluded that the wild pollinators were excluded from foraging on the richest food sources (e.g. Schaffer *et al*., 1979), in hybrid sunflower pollination interspecific interaction increased honeybee movement from male-fertile to male-sterile flower rows (Greenleaf and Kremen, 2006). As honeybee movement to another flower head enhances cross-pollination (Free, 1993; Nderitu *et al*., 2008), it is likely that fields surrounded by natural vegetation with multiple flower visitors provide better sunflower pollination service than fields with managed honeybee colonies only.

Implications for conservation and sunflower farming

Sunflower production benefits from diversified populations of flower visitors. Studies in different parts of the world have stated that greater diversity of wild pollinators can improve seed set (Steffan-Dewenter and Tscharntke, 1999), which is important to maintain sustainable crop pollination service (Kremen, 2008). Combined flower visitation from honeybees and wild bees results in better seed and fruit set than one species alone (Kremen, 2008). In accordance with previous results (Steffan-Dewenter *et al*., 2002; Ricketts *et al*., 2008), this study shows that both the abundance of wild pollinators (wild honeybees and other insects) and sunflower seed yield decreased with distance from natural vegetation. Such declines were expected since provision of mass flowering plants enhances the abundance of beneficial insects for pollination (Pontin *et al*., 2006). Previous studies show that the establishment of ecological compensation areas near to intensively managed agricultural farms is essential to maintain diverse pollinator communities and to benefit from sufficient pollination service (Albrecht *et al.,* 2007). Moreover, marginal habitats such as hedgerows and roadsides may also provide food resources in times when crops might not be in flower and nests for important pollinators (Hopwood, 2008; Vaughan and Black, 2008). Therefore, conservation of natural vegetation near sunflower farms as a source of wild honeybee colonies and other wild pollinators is recommended for sunflower producers. In addition, creation of ecological compensation areas will help to maintain pollination services in areas where there is no natural habitat.

Furthermore, landscapes with a higher proportion of organic crop fields provide the required amount of food resources to sustain greater abundance of pollinator species in intensively managed agricultural landscapes (Holzschuh *et al.*, 2008). This is because organic farming is free from using pesticides, herbicides and inorganic fertilizers (Bengtsson *et al.*, 2005).

Future research

Several studies have shown that wild pollinators are as efficient as honeybees in providing pollination service. For example, Monzón *et al.* (2004) investigated whether *Osmia cornuta* and honeybees are equally efficient in pear pollination. Evaluating the pollination effectiveness of wild pollinators on sunflower pollination is important. During this study this was not possible, due to the low abundance of wild pollinators, more detailed studies where sampling effort is focused on areas near natural habitat could help in evaluating the pollination effectiveness of wild pollinators on sunflower pollination.

Another research focus should be to estimate the population density of wild honeybee colonies surrounding sunflower fields. This will be helpful to estimate the potential of wild honeybee population for sunflower pollination.

Human activities such as habitat fragmentation, changes in land use, modern agricultural practices and the use of pesticides and herbicides which harm potential pollinators are increasing the susceptibility of regions worldwide to pollination limitations (Kearns *et al.*, 1998). To fully understand the contribution of anthropogenic sources for pollination disruption and to come up with an appropriate management strategy for sustainable pollination services, further research is necessary in South Africa.

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Appendix

Effect of isolation from natural habitat on the abundance of sunflower Lepidoptera pests

Introduction

Noctuidae are known as serious pests of sunflowers and have a negative impact on sunflower seed yield (Mitchell, 1984). This crop species is the most preferred host plant of *Helicoverpa armigera* Hübner (1809) (Noctuidae) for oviposition (Firempong and Zalucki, 1990). Oviposition of *H. armigera* is concentrated on the fruiting parts of sunflowers, which are the favoured feeding sites of larvae (Denberg and Cock, 1995). The objective of this preliminary study was to assess how the abundance of sunflower pests varied with distance from natural habitat, to evaluate the level of damage on sunflower plants in the presence or absence of natural enemies and to identify potential biological control agents.

Materials and methods

To test the effect of distance from natural habitat on sunflower pests, the abundance of Noctuidae was assessed on 32 plots located at different distances from natural habitat (30 to 1920 m) throughout 15 commercial sunflower fields in Settlers (27^0 57'S, $28^0$32'E). A total of 1074 sunflower plants were selected, of which 498 experimental flowers were covered with a nylon mesh bag before florets started opening and 576 control flowers were left open to be visited by natural enemies (e.g. predatory birds or insect parasitoids).

At the end of the flowering period, between 27 April and 01 May 2009, all bags were removed and the numbers of caterpillars found on bagged and open sunflowers was recorded, as well as the percentage of damaged flower heads. To investigate the variation in the number of emerging internal parasitoids between treatments, 113 caterpillars in a similar stage of development were collected and reared in lab conditions. Of these, 30 and 22 were from experimental sunflower heads located in the fields with managed hives and natural habitat respectively; 31 and 30 control sunflower heads from the fields with managed hives and natural habitat respectively were sampled for assessing parasitism rates.

Samples were maintained in a climate room (27-28^{0}C) in individual plastic boxes with mesh cloth covers. Caterpillars were supplied with fresh sepals, leaves and green immature soft seeds of sunflowers every two days for the first two weeks, and thereafter twice a week until they pupated. The feeding preference of caterpillars, date of pupation, number of parasitoids emerged from caterpillars and pupae, dead caterpillars and pupae, and the number of adult moths developed from pupae were recorded. Whenever a caterpillar died the individual was kept for three weeks to see if any parasitoid emerged. To accelerate moth emergence, the moth pupae were kept separately in the incubator (29^{0}C) until an adult moth emerged. Emerged adult moths were identified to species level at the Transvaal Museum in Pretoria and internal parasitoids were sent to taxonomists for identification. Caterpillars that were affected by diseases or escaped were not considered in data analysis.

Fisher's exact test was performed to evaluate if the abundance of internal parasitoids that emerged from caterpillars and pupae was significantly affected by bagging (bagged and open plot flowers). Furthermore, the effect of distance (distance was grouped as 1: 30 - 480 m, 2: 980, 3: 1400 -1500 m) on the abundance of parasitoids was analyzed using Kruskal-Wallis ANOVA by ranks test followed by multiple comparisons.

The effect of treatments (bagged vs. open pollinated flowers) on the abundance of Noctuidae was compared using one-way ANOVA with treatments (bagged *vs.* open pollinated flowers) as categorical independent variable. The effect of distance (distance was grouped as 1: 30 - 560, 2: 815 - 1200 and 3: 1400 - 1920 m) on the abundance of Noctuidae was tested using one-way ANOVA where only data from open sunflowers from fields near to natural habitat were used and followed by post-hoc comparison to compare groups.

The percentage damage by caterpillars to sunflower heads was analyzed using the Mann-Whitney U test with treatments (bagged vs. open pollinated flowers) as independent variables and percent damaged as dependent variable. In addition, the effect of distance on the level of sunflower damage due to caterpillar was analyzed using Kruskal-Wallis ANOVA by ranks test followed by multiple comparisons to evaluate if there was variation between different distances.

All statistical analyses were performed using Statistica 7.0 (1984 - 2004).

Results

Effect of distance on the abundance of sunflower pests and their parasitoids

Of the total 1074 sunflower heads selected for evaluating the distribution of caterpillars with distance from natural habitat, six experimental and 27 controls were lost due to damage. As a result a total of 492 experimental and 549 controls were used in this observation. Noctuidae abundance was significantly higher in plots far from natural habitat than in plots located near and at medium distance from natural margins ($F = 5.41$, $df = 2$, $P < 0.01$, see Table 1).

From the 176 noctuid caterpillars observed to feed and reproduce on sunflower heads, damaging large numbers of sunflower heads, 99.4% were *Helicoverpa armigera* Hübner (1809), subfamily Heliontinae and 0.6% were *Trichoplusia orichalcea* Fabricius (1775), subfamily Plusiinae. In addition, 74 caterpillars (*H. armigera*) were observed in the laboratory for feeding preference on different parts of sunflower heads by supplying equal amounts. Thirty-three (44.6%) of them were feeding on sepals and 9 (12.2%) on leaves of sunflower. Nineteen (25.7%) were feeding on sepals and leaves, three (4%) on sepals and green immature soft seeds and the remaining 10 (13.5%) were feeding on sepals, green immature soft seeds and leaves of sunflower.

The abundance of Noctuidae was significantly higher in bagged flowers than in open flowers ($F = 51.88$, $df = 1$, $P < 0.0001$, see Table 2).

Emergence of parasitoids

A total of 40 parasitoids (Tachinidae: *Exorista* sp? and Ichneumonidae) emerged from the 82 caterpillars and pupae collected from bagged (n = 40 caterpillars) and open (n = 42 caterpillars) sunflower heads. The number of parasitoids emerged from caterpillars and pupae in the open sunflowers (n = 30 parasitoids) was significantly higher than the number of parasitoids in the bagged sunflowers (n = 10 parasitoids) (Fisher's exact test, $P < 0.0001$). The overall model shows there was a significant difference in the abundance of parasitoids with distance from natural habitat (Kruskal-Wallis ANOVA by ranks test: $H_{2, 82} = 8.19$; $P < 0.02$). In addition, multiple comparisons shown that there was a significant difference in the abundance of parasitoids between different distances (Table 3).

Damage to sunflower crops

Although low, the percentage of damaged sunflower seeds in the bagged plots (0.86 ± 1.90 %) was significantly higher than in the open plots (0.15 ± 0.45 %) (Mann-Whitney *U* test: z = -6.30; $P < 0.0001$). The overall model shows there was a significant difference in the level of sunflower damage due to caterpillars between different distances from natural habitat (Kruskal-Wallis ANOVA by ranks test: $H_{2, 549} = 13.16$; $P < 0.002$). However, multiple comparisons did not explain the variation between different distances.

Discussion

Helicoverpa armigera is known as one of the major agricultural pests (Cunningham *et al.*, 1999), which destroys sunflower crops (Kakakhel *et al.*, 2000). This preliminary study showed that *H. armigera* was a major sunflower pest in Settlers commercial farms. The distribution of Noctuidae moth caterpillars was found to be positively correlated with distance from natural habitat and with bagging of sunflower heads. However, the distribution of internal parasitoids was positively correlated with distance from natural habitat and inversely correlated with bagging sunflowers. Therefore, the possible reason to have more caterpillars at a greater distance might be due to less predation from natural habitat, whereas the high abundance of caterpillars in bagged flowers could be due to both less predation and parasitism. This interpretation is supported by significantly higher emergence of parasitoids (Tachinidae and Ichneumonidae) from open flowers compared to bagged flowers. Accordingly, parasitoids of Tachinidae and Ichneumonidae are advantageous as biological control for major sunflower pests in Settlers commercial farms. A previous study also stated that the effect of parasitism of *H. armigera* by Ichneumonidae was higher on sunflower plants than on chickpea (Murray et al., 1995). Furthermore, the abundance of *H. armigera* was found to be higher where crawling predators were excluded from sunflower heads (Denberg *et al*., 1997). Although there was no significant difference between different distance points, the trend shows that the level of sunflower head damage due to caterpillars increased with distance from natural habitat. Furthermore, detailed future study is recommended on the infestation level of Noctuidae on sunflowers, the effect on seed yield and the possible control strategy.

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Appendix (Table 1). Results of post-hoc analysis (Tukey HSD test) comparing the effect of distance from natural habitat on the abundance of Noctuidae on sunflower heads.

Distance from natural margin	Number of Noctuidae per flower head (means ± SD)	n
Near (30-560 m)	$0.59^{a} \pm 1.06$	242
Medium(815-1200 m)	$0.59^{a} \pm 0.79$	161
Far (1400-1920 m)	$0.89^{b} \pm 0.88$	146

Different letters in the column indicates significant difference between treatment means at $P < 0.02$

Appendix (Table 2). Results of post-hoc analysis (Tukey HSD test) comparing the effect of bagging sunflower heads on the abundance of Noctuidae.

Treatment	Number of Noctuidae per flower head (means ± SD)	n
Open flowers	$0.59^{a} \pm 1.06$	242
Bagged flowers	$1.52^{b} \pm 1.66$	218

Different letters in the column indicates significant difference between treatment means at $P < 0.0001$

Appendix (Table 3). Results of multiple comparisons showing the effect of distance from natural habitat on the abundance of internal parasitoids.

Distance from natural margin	Number of internal parasitoids per flower head (means ± SD)	n
Near (30 - 480 m)	$0.33^{a} \pm 0.76$	30
Medium(980 m)	$0.38^{ab} \pm 0.65$	13
Far (1400-1500 m)	$0.64^{b} \pm 0.58$	39

Different letters in the column indicates significant difference between treatment means at $P < 0.05$